Investigating the Bioactive Potential of *Persicaria hydropiper*: GC-MS Profiling and In Vivo Exploration of Antinociceptive and Antidiarrheal Effects

ABSTRACT

Aims: This study aimed to investigate the impact of methanol-derived leaf extracts from the *Persicaria hydropiper* plant on the GCMS analysis and in-vivo antinociceptive and antidiarrheal activities.

Study Design: The GCMS analysis was used to analyze the phytochemicals of the methanolic extract of *Persicaria hydropiper* (MEPH). The research aimed to investigate the possible in-vivo activities, including the antinociceptive and antidiarrheal activity, of the plant's chemical ingredient, which is of pharmaceutical significance. Whether the changes seen in experimental animals have statistical significance.

Methodology: Potential antinociceptive and antidiarrheal properties of MEPH were studied after phytochemicals were found by GCMS analysis of the plant. Swiss albino mice assessed antidiarrheal activity using the castor oil-induced method and antinociceptive activities at various dosages using the hotplate and glutamate-induced nociception methods, respectively.

Results: The MEPH GCMS analysis revealed that 65 phytochemicals were found which have greater pharmacological activities. In contrast, MEPH inhibited peripheral nociception in the glutamate-induced paw licking nociceptive paradigm with percent inhibitions of 86.53 and 93.59, respectively. In addition, the hot plate test revealed a significant antinociceptive effect. Where the castor oil-induced antidiarrheal method showed 80.16 and 87% of inhibition of diarrhea compared to the standard loperamide's value of 84.19%. Each pharmacological model was experimented using the dose of 200 and 400 mg/kg.

Conclusion: Several pathological conditions, including dysentery, Persistent diarrhoea, arthritis and other pain, inflammation related diseases, may benefit in the future from the use of plant-derived pharmacological agents due to their antinociceptive and antidiarrheal activities.

Keywords: *Persicaria hydropiper*, antinociceptive, antidiarrheal, GCMS, phytochemicals

INTRODUCTION

Persicaria hydropiper Linn., a member of the Polygonaceae family, is a widely found plant in Bangladesh, often referred to as Bishkatali. *Persicaria*, a clade within the Polygonaceae family, thrives in the vicinity of rivers, canals, lakes, and roadsides in tropical and sub-tropical regions [1]. Leaves in traditional medicine are used to alleviate rheumatic pain, knee pain, gout, and skin diseases like ringworms, scabies, boils, abscesses, carbuncles, snake, dog or bug bites, colic pain, diuretic effects, and amenorrhea. Occasionally, the leaves are used as a condiment or additive for enhancing taste [2]. Polygonums are regarded as an optimal source of sustenance during times of famine due to their abundant availability and significant nutritional content. They are projected to serve as a significant resource in addressing nutritional

deficiencies during periods of food shortage [3]. Various varieties of *Persicaria* have yielded a diverse array of flavonoids, terpenoids, anthraquinones, apianen lactones, and steroids. Several bioactive compounds with anticancer, antioxidant, antinociceptive, antileukemic, antibacterial, and tyrosinase-inhibitory properties have been identified in different *Persicaria* species [4]. The herb has been shown to have bitter, stimulant, tonic, diuretic, carminative, emmenagogue, haemostatic, and lithotriptic effects. The plant is used for the treatment of many conditions such as diarrhea, dyspepsia, pruritus, excessive menstrual flow, and hemorrhoids, either in isolation or in combination with other herbal remedies[5].

The GCMS analysis, antinociceptive and anti-diarrheal effects, and organic soluble component profiles of a whole plant methanol extract were the subjects of this investigation. The plant's methanolic leaf extract was studied for these purposes.

2.METHODS

2.1 Plant Material

The plant *P. hydropiper* was taken from West Delpara Road in the Narayanganj area of Dhaka, Bangladesh in September 2023 during daylight hours. The plant was identified by scientists from the Bangladesh National Herbarium in Mirpur, Dhaka. It has been assigned the accession number DACB 39853 and a voucher specimen has been placed in the Pharmacy Department at Stamford University Bangladesh.

2.2 Preparation of the plant extract

The dried leaves shed were ground into a fine powder using a commercial grinder. Then, about 170 g of each powered material was macerated with 900 mL of methanol in a beaker at 23 ± 2°C for 72 hours, stirring every 18 hours with a sterile glass rod[6]. Alkaloids, glycosides, tannins, flavonoids, steroids, and so on are only a few chemical classes in the methanol used to extract plant bioactive components. The boiling point of methanol is lower than that of other organic solvents, and its polarity index is 5.1. The whole combination was then coarse-filtered using a sterile, white cotton filter cloth. Filter paper from Whatman 102 was used to collect them. The filtrates were evaporated using a rotary evaporator manufactured by Shanghai Biochemical Equipment Co., Ltd. (BC-R 201). We got 27.6 g of dried extract (a yield of 19.89%) from 170 g of powder after drying. Analgesic and antidiarrheal activity experiments were conducted on the methanol extract of P. hydropiper using gas chromatography-mass spectrometry[1].

2.3 Experimental animals

Mice ranging in weight from 20 to 26 g were chosen for this study because they were young and in good condition.International Center for Diarrheal Disease and Research (ICDDR) of Bangladesh, was the supplier of the mice. It was critical to maintain things

as they were. A typical day in this part of the world would see a temperature of 77 degrees Fahrenheit, a relative humidity of 55 to 65 percent, and a 24-hour cycle of light and dark. Conditions are kept constant for 8 days after collection. Following the advice of the ICDDRB, mice were provided with a diet of sufficient food and clean water to help them recover from the water and food deprivation they suffered during transit and to help them adjust to the laboratory setting. The mice were prepared for the experiment after a week of rest.

2.4 GC-MS (Gas Chromatography-mass Spectroscopy) Analysis of MEPH

The normal protocol was followed to conduct GC-MS (Gas chromatography-mass spectroscopy) analysis on a newly prepared MEPH. Agilent Technologies' 7890A capillary gas chromatograph was used for the GC-MS analysis; the mass spectrometer equipment was a 5975C inert XL EI/CI MSD with a triple-axis detector. The HP-5MSI capillary column, which is composed of 5% phenyl and 95% dimethyl-poly-siloxane, has the following dimensions: length: 90 m, diameter: 0.250 mm, and film: 0.25 µm. Here is how the GC parameters were set: the intake temperature was set at 250oC and the oven temperature was programmed as follows: 90^{o}C for 0 minutes, 3^{o}C/min to 200^{o}C for 2 minutes, and then 15^{o}C/min to 280^{o}C for 2 minutes. The column flow rate was 1.1 mL/min of helium gas, and the total run duration was 46 minutes. 280°C was chosen as the auxiliary temperature for the GC to MS interface. With the MS in scan mode, the MS parameter was set[7], [8].

2.5 Analgesic studies

2.5.1 Hot plate test

Mice who exhibited behaviors such as licking their forepaws, withdrawing their paw(s), or leaping reaction within 15 seconds on a hot plate maintained at 51 ± 0.5°C were chosen for this investigation 24 hours before the beginning of the experiment. The mice were allowed to fast overnight and were given water as needed. Following administration of morphine or MEPH, the animals were put on Eddy's hot plate, which was maintained at a temperature of 51 ± 0.5°C. In order to prevent harm to the paw tissue, a cutoff duration of 20 seconds was maintained [9], [10]. At30,60,90, and 120 minutes after treatment, we observed the reaction as forepaw licking, paw withdrawal, or leaping.

2.5.2 Glutamate-induced nociception

A volume of 20 microliters of glutamate solution, containing 10 micromoles per paw, was administered to the underside of the right hind paw of mice. This was done 30 minutes after MEPH therapy and 15 minutes after diclofenac sodium injection. The mice were

monitored for 15 minutes after the administration of glutamate, and the act of licking their paws was recorded as a measure of pain perception[11], [12].

2.6 Antidiarrheal test

Mice of both sexes were fasted for 18 hours before to the experiment. All procedures used in the experiment were standard operating procedures [13]. Each of the eleven groups of six mice used in the experiment served as an independent cohort. The first set of subjects called the control group, received 5 mL/kg of a vehicle consisting of 1% Tween-80 in normal saline. The second group was given loperamide orally at a dosage of 3 mg/kg body weight as the standard medicine; this was called the positive control group. The experimental groups were given several types of extracts: aqueous extract to groups 3, 4, and 5, methanol extract to groups 6, 7, and 8, and oral doses of 50, 100, and 200 mg/kg body weight of MEPH extracts to groups 9, 10, and 11, respectively. One hour later, 2 g/kg of magnesium sulfate was orally administered to every mouse.On top of the white filter paper, each item was placed in its container. Over 240 minutes, the frequency of loose or unformed stool during defecation was recorded.

The formula for detecting antidiarrheal properties of MEPH are given below:

$$\% \text{ of fecal output} = \left(\frac{Mean\ \ fecal\ \ weig\,ht\ of\ \ eac\,h\ treatment\ \ \ group}{Mean\ \ fecal\ \ weig\,ht\ of\ \ control\ \ \ group}\right) \times 100\ \%$$

$$\text{inhibition of defecation} = \left(\frac{M1-M}{M1}\right) \times 100$$

Where,

M1: Mean defecation of control, M: Mean defecation of test sample/standard drug.

2.7 Statistical analysis

The data shown in the table is the mean value of all three replicates of the bioassay. We used Excel to do our statistical analysis.

3. Results

3.1Antinociceptive activities

3.1.1 Hot plate test

The results of the hot plate test showing the antinociceptive effects of MEPH and morphine are shown in Table 1. There was a significant increase in the response latency to the heat stimuli when administered dosages of 200 and 400 mg/kg of MEPH. We found a larger antinociceptive effect at 400 mg/kg compared to 200 mg/kg, indicating that the impact was dose-dependent. During every time point of observation, morphine exhibited the longest delay. At dosages of 200 and 400 mg/kg, the extract also significantly increased the latency to heat stimulation.

Table 1.Primary data table for hot plate test for plant extract of MEPH

Reaction time at different time intervals (in sec)					
Group	**Average wt. of mice (g)**	**30 min**	**60 min**	**90 min**	**120 min**
Control		6.5±0.1	7.4±0.2	6.2±0.2	5.3±0.1
Morphine (5mg/kg)		8.5±0.3	9.3±0.1	11.2±0.1	6.8
MEPH (200mg/kg)	20 to 26	8.9±0.3	11.9±0.2	14.7±0.1	0
MEPH (400mg/kg)		13.1±0.1	0	0	0

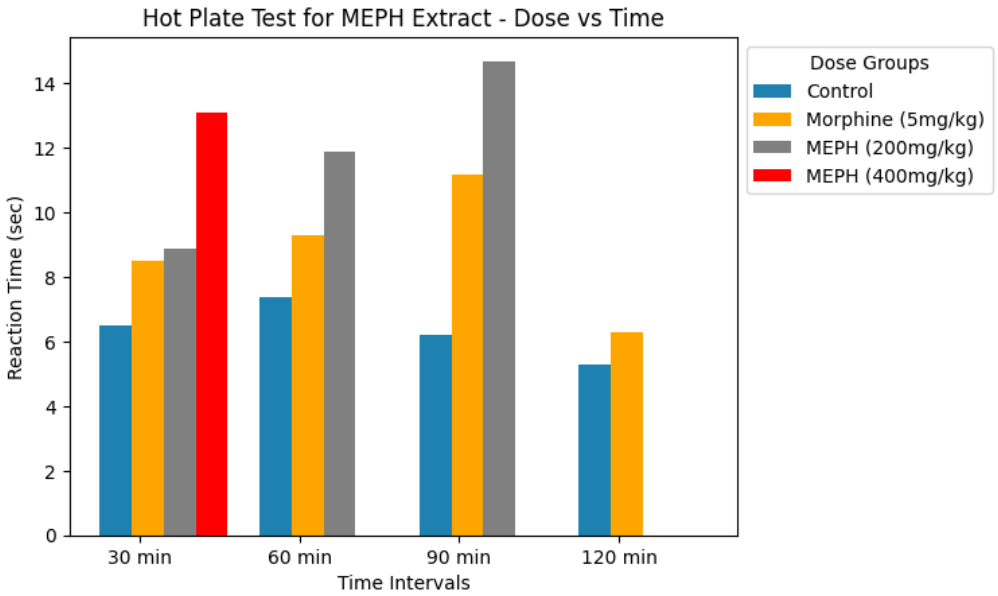

Figure 1: Graph of hot plate test

3.1.2 Glutamate-induced nociception

Two dosages of oral MEPH, 200 and 400 mg/kg inhibited glutamate-induced nociception by 86.52% and 93.59%, respectively. The standard medication group had a 91% inhibition when compared to the control group using diclofenac sodium (10 mg/kg). There was a statistically significant impact across all treatment groups (Table 2).

Table 2. Effect of leaf extract of *P. hydropiper* extract in glutamate-induced nociception

Administered drug	Dose	Licking time	% Of Inhibition
Control	10mL/kg	169.2 ± 4.75	0.00
Diclofenac sodium	10mg/kg	12.18 ± 1.71	91.0
MEPH	200mg/kg	22.2 ± 1.13	86.52
MEPH	400mg/kg	9.72 ± 2.15	93.59

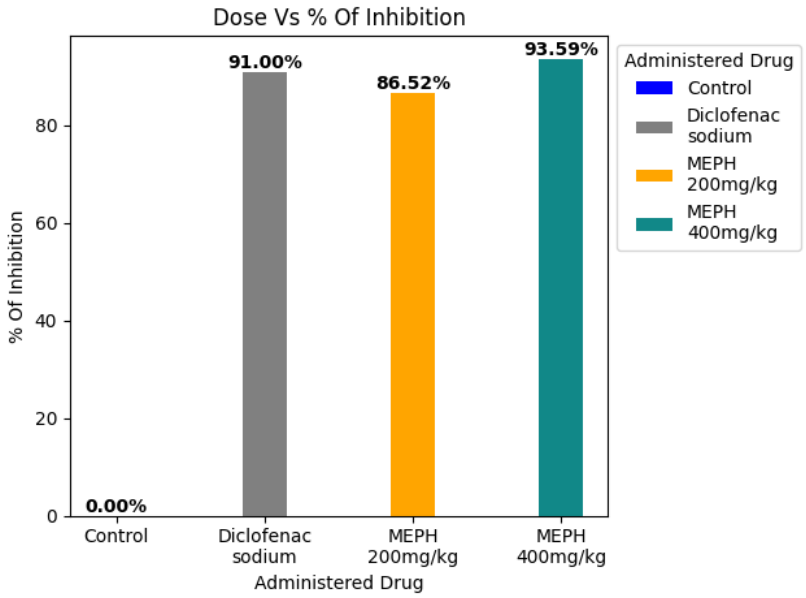

Figure 2: Graph of glutamate induced nociception.

3.2 Antidiarrheal activities

The results of the effect of MEPH on magnesium sulfate-induced diarrhea are shown in Table 3. All of the extracts showed statistically significant suppression of diarrhea at 200 and 400 mg/kg compared to the control group. Dosages of 10 mg/kg of loperamide

inhibited diarrhea by 84.19%, respectively, after four hours after delivery. At 200mg/kg and 400 mg/kg, the comparable values for the MEPH are 80.16% and 87%. The observed order of diarrhea and feces inhibition is quite potent.

Table 3: Antidiarrheal effects of MEPH

Administered drug	Dose	% inhibition of defection	% Of Inhibition
Control	10mL/kg	0.00	0.00
Loperamide	10mg/kg	81.26	84.19
MEPH	200mg/kg	52.37	80.16
MEPH	400mg/kg	63.06	87.00

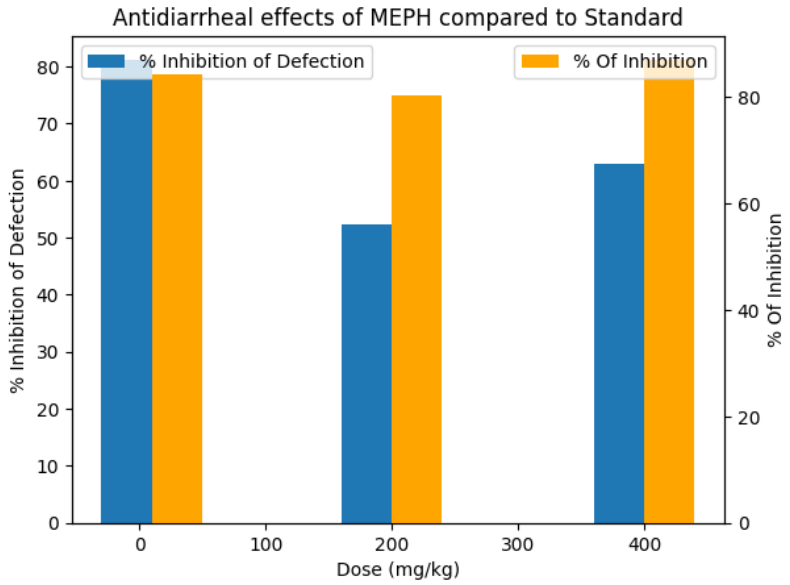

Figure 3: Graph of antidiarrheal test

3.3 GC-MS analysis

Table 4 and Figure 1 demonstrate that 64 bioactive chemicals, including major and minor compounds, were found in the methanol fraction of the PH sheet, according to the GCMS analysis.

Table 4 Identification of the compounds by GC-MS method and their biological activity

Sl.No	Ret. Time	Compounds Name	Molecular formula	Molecular weight (g/mol)	Nature of the compound
1	3.745	Benzyl alcohol, benzyldimethylsilyl ether	$C_{16}H_{20}OS$	256.420	Organic compound
2	4.81	Methyl 2,2-dimethyl-3,6,9-trioxa-2-silaundecan-11-oate	$C_8H_{20}O_3Si$	192.33	Organic compound
3	4.875	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	144.12	Organic compound
4	4.975	1,2,3-Butanetriol	$C_4H_{10}O_3$	106.12	Hydroxyl compound
5	9.625	N-Propionyl-D-glucosamine	$C_8H_{15}NO_6$	221.21	Organic compound
6	10.71	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	$C_{11}H_{16}O_2$	180.2435	volatile terp ene organic
7	11.195	Cholestane, 4,5-epoxy-, (4.alpha.,5.alpha.)-	$C_{27}H_{46}O$	386.7	Organic compound
8	11.405	Spiro[(tricyclo[6.2.2.0(2,7)]dodeca-5,9-diene)-4,1'-cyclobutane]-11,2'-dione, 1,3,3,5,12,12-hexamethyl-	$C_{21}H_{28}O_2$	312.44582	Organic compound
9	11.465	4a,5-Dimethyl-3-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,7-octahydronaphthalen-1-ol	$C_{15}H_{24}O$	220.3505	Organic compound
10	11.655	Alloaromadendrene oxide-(1)	C15H24O	220.35	organic
11	11.74	Neoisolongifolene, 8-bromo-	$C_{15}H_{23}Br$	283.25	Organic compound
12	11.87	Isoaromadendrene epoxide	$C_{15}H_{24}O$	220.35	Organic compound

13	12.295	(-)-Globulol	$C_{15}H_{26}O$	222.37	Tricyclic hydroazulene sesquiterpene
14	12.57	Aromadendrene oxide-(1)	$C_{15}H24O$	220.35	Organic compound
15	12.77	3,5,5-Trimethylcyclohexyl ethylphosphonofluoridate	$C_{11}H_{22}FO_2P$	236.26	Organic compound
16	13.035	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	$C_{11}H_{16}O_3$	196.2429	Organic compound
17	13.12	Drim-7-en-11-ol	$C_{15}H_{26}O$	222.3663	Organic compound
18	13.195	(E)-2-((8R,8aS)-8,8a-Dimethyl-3,4,6,7,8,8a-hexahydronaphthalen-2(1H)-ylidene)propanal	$C_{15}H_{22}O$	218.33	Organic compound
19	13.56	7.alpha.-Methylthiotestosterone acetate	$C_{19}H_{28}O_2$	288.431	Steroid
20	13.68	Neophytadiene	$C_{20}H_{38}$	278.5	Sesquiterpenoids
21	13.765	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268.5	Organic compound
22	13.88	3,4-Hexadienal, 2-butyl-2-ethyl-5-methyl-	$C_{13}H_{22}O$ [42]	194.31	Organic compound
23	13.93	Cyclohexene, 1,5,5-trimethyl-6-acetylmethyl-	$C_{12}H_{20}O$	180.29	Organic compound
24	14.005	3,7,11,15-Tetramethyl-2-hexadecen-1-ol (phytol)	$C_{20}H_{40}O$	296.539	Acyclic diterpene alcohol
25	14.77	Methyl hexadec-9-enoate (Palmitoleic acid)	$C_{17}H_{32}O_2$	268.4	Omega-7 monounsaturated fatty acid
26	14.84	Hexadecanoic acid, methyl	$C_{17}H_{34}O_2$		Fatty acid methyl ester

		ester (Methyl palmitate)		270.5	
27	14.975	(1aR,4S,4aR,7R,7aS,7bS)-1,1,4,7-Tetramethyldecahydro-1H-cyclopropa[e]azulen-4-ol (Epiglobulol)	$C_{15}H_{26}O$	222.3663	Non-polar
28	15.35	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	Saturated fatty acid
29	15.64	Aristolene epoxide	$C_{15}H_{24}O$	220.35	Oxygenated Sesquiterpenoids Terpenoid
30	15.94	Bicyclo[5.1.0]octan-2-one, 4,6-diisopropylidene-8,8-dimethyl-	$C_{16}H_{24}O$	232.36	Ketone
31	16.26	Cyclooprop[e]indene-1a,2(1H)-dicarboxaldehyde, 3a,4,5,6,6a,6b-hexahydro-5,5,6b-trimethyl-, (1a.alpha.,3a.beta.,6a.beta.,6b.alpha (Isovelleral)	$C_{15}H_{20}O_2$	232.32	Sesquiterpene
32	16.31	Cycloheptane, 4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-	$C_{15}H_{24}O_2$	204.35	Organic compound
33	16.39	(1R,4aS,6R,8aS)-8a,9,9-Trimethyl-1,2,4a,5,6,7,8,8a-octahydro-1,6-methanonaphthalen-1-ol (Norpatchouleno)	$C_{14}H_{22}O$	206.32	Tri cyclic terpenoid
34	16.665	Benzo[e]isobenzofuran-1,4-	$C_{15}H_{20}O_3$	248.32	Organic compound

		dione,1,3,4,5,5a,6,7,8,9,9a-decahydro-6,6,9a-trimethyl			
35	16.73	(2R,3R,4aR,5S)-2-Hydroxy-4a,5-dimethyl-3-(prop-1-en-2-yl)-3,4,4a,5,6,7-hexahydronaphthalen-1(2H)-one	$C_{15}H_{22}O_2$	234.3340	Organic compound
36	17.235	1-Naphthalenemethanol, 1,4,4a,5,6,7,8,8a-octahydro-2,5,5,8a-tetramethyl-	$C_{15}H_{26}O$	222.37	Versatile synthon
37	17.41	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294.5	Polyunsaturated omega-6 fatty acid
38	17.52	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292.4562	Polyunsaturated omega-6 fatty acid
39	17.7	Phytol	$C_{20}H_{40}O$	296.539	Acyclic diterpene alcohol
40	17.875	Methyl stearate	$C_{19}H_{38}O_2$	298.5	Fatty acid methyl ester and an octadecanoate ester
41	17.94	6,6-Dimethylhepta-2,4-diene	C_9H_{16}	124.22	Organic compound
42	18.02	9,12-Octadecadienoic acid (Z,Z)-	$C_{19}H_{34}O_2$	294.5	polyunsaturated omega-6 fatty acid
43	18.37	.beta.-Cyclocostunolide	$C_{15}H_{20}O_2$	232.32	Sesquiterpene lactones
44	18.425	3-Buten-2-ol, 3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-	$C_{14}H_{24}O$	208.34	Organic compound
45	18.47	Aristolene epoxide	$C_{15}H_{24}O$	220.35	Oxygenated Sesquiterpe

					noids Terpenoid
46	19.105	(3E,5E,7E)-6-Methyl-8-(2,6,6-trimethyl-1-cyclohexenyl)-3,5,7-octatrien-2-one	$C_{18}H_{26}O$	258.4	Organic compound
47	19.45	Succinic acid, dodec-2-en-1-yl 2-ethylphenyl ester	$C_{24}H_{36}O_5$	404.5396	Organic compound
48	19.525	2H-Cyclohepta[b]furan-2-one, 6-[1-(acetyloxy)-3-oxobutyl]-3,3a,4,7,8,8a-hexahydro-7-methyl-3-methylene-	$C_{17}H_{22}O_5$	306.3536	Organic compound
49	23.975	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{19}H_{38}O_4$	330.5026	Amino compound
50	26.72	n-Propyl 9,12-octadecadienoate (Propyl linoleate)	$C_{21}H_{38}O_2$	322.5	Fatty acid ester
51	26.85	Butyl 9,12,15-octadecatrienoate	$C_{22}H_{38}O_2$	334.5	Organic compound
52	27.125	Octadecanoic acid, 2,3-dihydroxypropyl ester	$C_{21}H_{42}O_4$	358.5558	Fatty Acid Esters
53	28.08	13-Docosenamide, (Z)-(Erucamide)	$C_{22}H_{43}NO$	337.6	Amide of docosenoic acid
54	29.72	Propyl tetratriacontyl ether	$C_{37}H_{76}O$	537	Organic compound
55	29.85	Tetracosamethyl-cyclododecasiloxane	$C_{24}H_{72}O_{12}Si_{12}$	889.8	Organic compound
56	32.385	Cholesta-4,6-dien-3-ol,	$C_{27}H_{42}O$	382.6	Oxidation of cholesterol

		(3.beta.)-			
57	32.49	1-Hexacosanol	$C_{26}H_{54}O$	382.7	Fatty alcohol
58	32.665	Tetracosamethyl-cyclododecasiloxane	$C_{24}H_{72}O_{12}Si_{12}$	889.8	Organic compound
59	33.06	Vitamin E (Tocotrienols)	$C_{29}H_{50}O_2$	430.7	
60	34.525	Campesterol	$C_{28}H_{48}O$	400.7	Steroid derivative
61	35.025	Stigmasterol	$C_{29}H_{48}O$	412.7	Steroid derivative
62	36	Gamma.-Sitosterol	$C_{29}H_{50}O$	414.7	Phytosterols
63	36.185	Phytonadione (vit k1)	$C_{31}H_{46}O_2$	450.70	Polycyclic aromatic ketone
64	36.98	Stigmast-7-en-3-ol, (3.beta.,5.alpha.,24S)-	$C_{29}H_{50}O$	414.7	Phytosterols

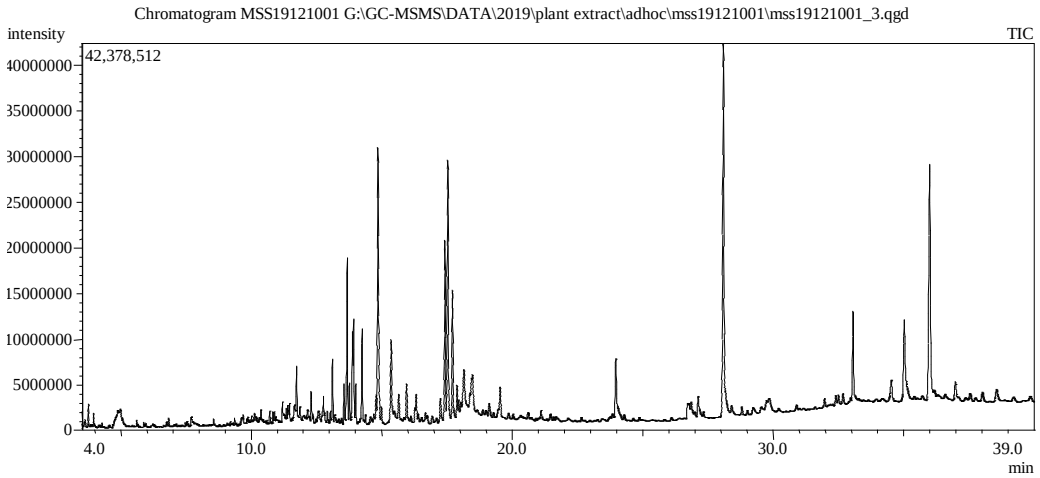

Figure 1. Chromatogram of GCMS analysis

4. Discussions

Between 4.0 and 39.0 minutes, 66 chemical components were identified using GC/MS analysis of the ethanolic extract, which relies on comparing retention indices and mass spectra (Fig. 1, Table 4).

The antinociception effect of MEPH has been assessed using several animal models of thermaland chemical nociception. When evaluating models of central and peripheral activity, the hot plate and glutamate-induced nociception tests have traditionally been used.A statistically significant antinociceptive effect of MEPH was shown by the results of the hot plate test.

In the hot plate test, the reversal effect was just as effective as the one against morphine. According to these findings, opioid receptors located in the spinal and supraspinal levels may be responsible for MEPH's antinociceptive effects[14]. The hot-plate test involves testing the animal's reaction to heat stimuli; the hotplate test measures a supraspinal reflex. Supporting this idea, research has shown that v2- and δ-opioid receptors play a role in the spinal mechanism, but μ1/μ2-opioid receptors may primarily mediate analgesia that occurs outside of the spinal cord [15], [16]. Thus, it is reasonable to assume that μ-opioid receptors may play a significant role in the central antinociceptive impact of MEPH.

A dose-dependent reduction of the nociceptive response in mice was seen after oral administration of MEPH, which was induced by injection of glutamate into the hind paw. According to reports, the nociceptive response that is triggered by glutamate is significantly influenced by sites of action with glutamate receptors, such as AMPA, kainate, and NMDA receptors [17], [5].

This provides further evidence that PKC (Protein Kinase C) also influences the glutamatergic system. This suggests that the connection between the glutamatergic system and MEPH is responsible for its antinociception effects[18].

Besides, Vitamin E,Campesterol,Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, 9,12,15-Octadecatrienoic acid, (Z, Z,Z)-, n-Hexadecanoic acid, Methyl palmitate, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (phytol), Neophytadiene, may contribute to the antinociceptive action due to their anti-inflammatory and antioxidative effects[19].

The previous research found that the antisecretory and antimotility mechanisms of the plant extract were linked to a reduction in stool frequency. These processes, in turn, reduce the amount and weight of wet stools[20]. It is believed that the extract contains phytochemicals like tannins and flavonoids, as shown by the dose-dependent delay in

defecation and the highest percentage reduction of wet feces at 400 mg/kg, which was 87% compared to the negative control[21].

The GC-MS analysis of MEPH revealed the presence of polysaccharides, polyphenols, amino acids, steroids, fatty acid esters, and triterpenoids such as 7.alpha.-methylthiotestosterone acetate, neophytadiene, palmitoleic acid, methyl palmitate, norpatchouleno, methyl stearate, aristolene epoxide, propyl linoleate, gamma.-sitosterol, stigmasterol, campesterol. These phytoconstituents also have antidiarrheal properties[22].

The mechanism might be considered to inhibit the production of prostaglandins produced by castor oil[23]. Previous research papers revealed that castor oil triggers diarrhea by releasing nitric oxide, which in turn enhances the permeability of the gastrointestinal membrane to calcium. This process stimulates the synthesis of prostaglandins, leading to an increase in the flow of fluid and electrolytes into the lumen of the bowel, consequently promoting peristalsis[24].

5. CONCLUSION

The contemporary scientific assessment of plants and herbs primarily focuses on confirming the traditional use of plants and determining the active constituents of extracts and preparations. Therefore, more investigation of conventional botanical remedies is necessary to establish the scientific foundation for their activity, effectiveness, and safety.

The current study's findings indicate that the crude methanolic leaf extract of Persicaria hydropiper has antinociceptive and antidiarrheal properties.
The observed effects exhibited statistical significance when administered at greater dosages. The mechanisms of these activities require additional investigation with various antagonists considering these findings. It appears highly plausible that Persicaria hydropiper, which contains a sedative agent, can be utilized as an antinociceptive at high doses. This may contribute to the development of a novel natural product with antinociceptive properties.

The study will further examine the various fractions of Persicaria hydropiper and provide a detailed analysis of the structural composition of the relevant components. The observation's results also bolster and validate the conventional application of various medical conditions.

9. ETHICAL APPROVAL AND CONSENT

This study adhered to all regulations established by the US Food and Drug Administration, the Declaration of Helsinki, and the International Conference on Harmonization. The Faculty of Science at Stamford University Bangladesh reviewed and approved the study methodology and obtained signed permission using reference

number SUB/ERC/202307. All participants in the research were required to provide a signed permission form and had the prerogative to withdraw at any time.

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